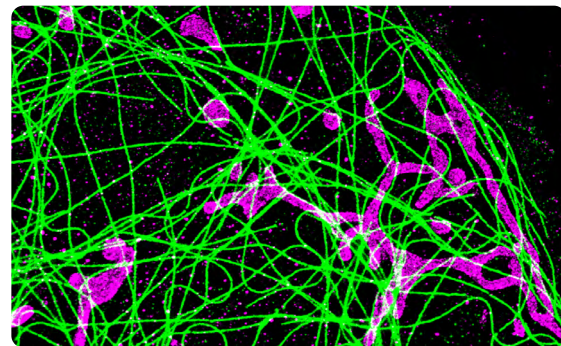


# Super-Resolution Microscopy

Fluorescent dyes and stains ideal for STORM and other super-resolution techniques



## Superior Dyes for Super-Resolution Microscopy

Super-resolution microscopy (SRM) encompasses diverse techniques that rely on extremely precise control over the excitation, emission, and image acquisition of fluorescently labeled cells and tissues. Consequently, the quality and efficiency of SRM relies heavily on the properties of the fluorescent dyes used. Biotium has met this demand with industry-leading CF® Dyes developed and validated for STORM and other SRM techniques (see page 3). CF® Dyes are offered in a wide array of products useful for SRM, including labeled primary and secondary antibodies, single-domain VHH conjugates (Nanobodies®), antibody labeling kits, reactive dyes, single-label secondary antibody conjugates for STORM, and CF® Dye conjugates of phalloidin, lectins, nucleotides, and more (see page 2 for more details). Biotium also offers a variety of stains for cell nuclei, extracellular vesicles, and other organelles that have been validated for use in SRM (see page 4).

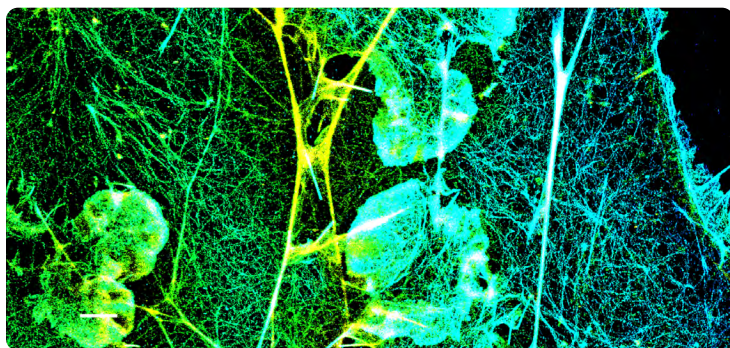


Figure 1. Typical (d)STORM image of the actin cytoskeleton in a fixed COS-7 cell labeled by phalloidin-CF®583R. (d)STORM was performed under standard conditions using a Tris-based (d)STORM buffer containing 100 mM cysteamine and an oxygen scavenger. Image courtesy of Bowen Wang, Michael Xiong, and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

## CF® Dye Advantages for Super Resolution

- Superior brightness and photoswitching properties
- Validated for STORM, 2-photon, TIRF, and more
- Selection of reactive dyes, including click chemistry
- Many color options available from blue to near-IR
- Unique dye options designed specifically for STORM

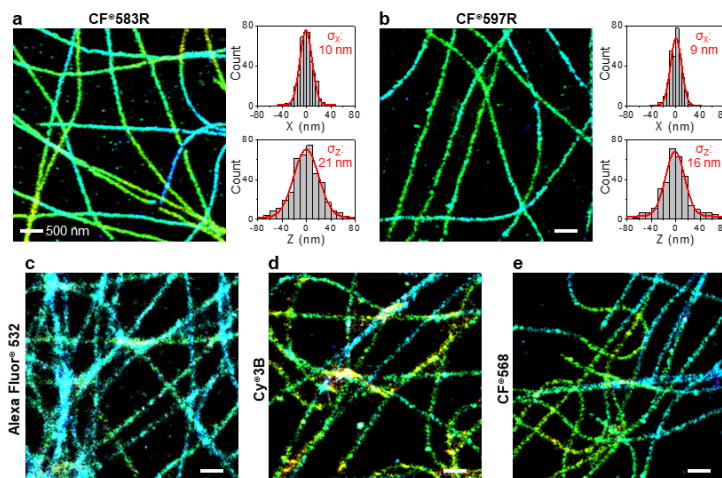


Figure 2. Comparison of typical (d)STORM images obtained in fixed COS-7 cells for microtubules immunolabeled by different dyes. (a) CF®583R. (b) CF®597R. (c) Alexa Fluor® 532. (d) Cy®3B. (e) CF®568. Localization distributions are given for single CF®583R and CF®597R molecules in the sample, in the X (in-plane; top) and Z (depth; bottom) directions, respectively. Gaussian fits (red curves) give standard deviations of ~10 and ~20 nm in the two directions, respectively. Color denotes depth (Z) values. Images and figures courtesy of Bowen Wang, Michael Xiong, and Professor Ke Xu, College of Chemistry, University of California, Berkeley.

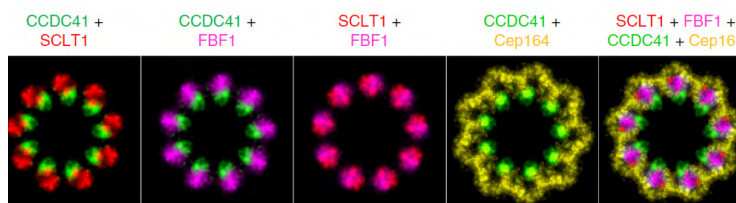


Figure 3. STORM analysis of centriole assembly. RPE-1 Centrin 1-GFP cells were labeled using primary antibodies for the indicated proteins and Biotium's CF®647 F(ab')2 secondary antibody conjugate then imaged using 3D STORM (pseudo-colored for display purposes). For scale, image edges represent approximately 650 nm across. Credit: Bowler et al. [doi.org/10.1038/s41467-018-08216-4](https://doi.org/10.1038/s41467-018-08216-4) reproduced under CC BY 4.0.

## CF® Dyes Validated for STED and FLIM Imaging

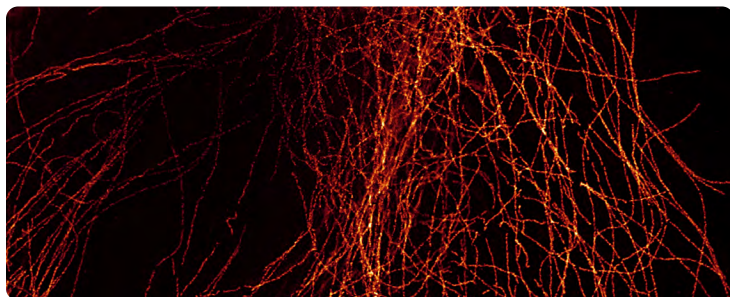


Figure 4. STED imaging of microtubules in U-2 OS cells. Microtubules were labeled with mouse anti-tubulin (DM1a) and anti-mouse CF®640R secondary antibody. Image acquired on a STELLARIS 8 STED FALCON confocal microscope, courtesy of Leica Microsystems GmbH, Germany.

## CF® Dye Products Useful for Super-Resolution

Label antibodies with one of Biotium's best-in-class CF® Dyes for STORM in 30 minutes using Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits, no purification needed. These kits produce a low 1–2.5 DOL (degree of labeling) ideal for STORM. Options also available for labeling Nanobodies® by conventional labeling or thiol labeling of Nanobodies® that have a free cysteine residue.

### Antibody Labeling Kits for STORM

- **Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits:** Optimized to yield 1-2.5 degree of labeling
- **Mix-n-Stain™ Nanobody Labeling Kits:** Options for conventional labeling or thiol labeling of cysteine residues
- **Validated Labels:** Best-in-class CF® Dye for STORM

## Single-Label Secondary Antibody Conjugates for STORM

Secondary antibodies with a low DOL have been reported to be optimal for STORM. Biotium offers single-label secondary antibody conjugates with an average DOL of 1 for STORM applications.

## Primary Single-Domain Antibodies (SdAbs) for STORM

Biotium's growing collection of MiniMab™ SdAbs (also known as Nanobodies® of VHH camelid domains) are highly optimized conjugates that offer reduced localization error for super-resolution. Available conjugated to best-in-class CF® Dyes for STORM, with options for neuroscience-focused targets GFAP, SYT1, and VGLUT1.

## CF® Dye Fluorescence Lifetime Data

Measurements were made on a Stellaris 8 STED FALCON microscope courtesy of Leica Microsystems, Germany.

Dye	$\tau$ (ns) /free acid in PBS pH 7.4, $\epsilon$ (ns)	$\tau$ (ns) /S.Ab <sup>§</sup>
CF®405S	3.88 ± 0.05	–
CF®488A	4.11 ± 0.05	1.705
CF®568	3.66 ± 0.05	1.539
CF®594	–	1.746
CF®633	3.39* (in water)	3*
CF®640R	2.38 ± 0.05	1.557
CF®647	1.07 ± 0.05	1.195
CF®680	1.23 ± 0.05	1.277
CF®680R	1.22 ± 0.05	1.6
CF®750	0.58 ± 0.05	0.636
CF®790	0.39 ± 0.05	0.54

§ Fluorescence lifetime measurements of CF® Dye labeled anti-mouse secondary antibodies used for immunostaining microtubules in U2OS using mouse anti-tubulin (DM1a) and mounted in ProLong™ Diamond.

\*Lifetime data obtained via customer communication under different experimental conditions and imaging setup.

## Reactive CF® Dyes

CF® Dyes in a wide array of reactive chemistries for your preferred labeling technique can be found on our website. Succinimidyl esters (SE) are ideal for efficiently labeling antibodies, proteins, or other amine-containing molecules, while maleimide and MTS CF® Dyes selectively label thiols. We also offer aminoxy and hydrazide reactive CF® Dyes for labeling carbonyl groups or carbohydrates, and azides, alkynes, as well as BCN CF® Dyes for highly specific bioorthogonal labeling.

### Reactive CF® Dyes

- **High efficiency SE Dyes:** For labeling amines in antibodies and other proteins
- **Maleimide and MTS Dyes:** For labeling thiols
- **Click Chemistry:** Alkynes, azides, and BCN for bioorthogonal labeling

## Probes for Cytoskeleton and Other Cellular Structures

Biotium offers a wide variety of CF® Dye bioconjugates for labeling specific cellular structures. This includes phalloidin conjugates for F-actin labeling available in 16 CF® Dye options; Con A, WGA, PNA, and other lectin CF® Dye conjugates; streptavidin and biotinylated CF® Dye conjugates, and CF® Dye nucleotides.

## CF® Dyes Validated for Super-Resolution Microscopy

Dye	Abs/Em (nm)	STORM	STED	SIM	2-Photon	TIRF	Other Applications	Features
CF®405S	411/431			•				• Brighter than Alexa Fluor® 405
CF®405M	416/452		•	•	•			• More photostable than Pacific Blue™ • Excellent choice for SIM imaging
CF®440	433/512		•					• More photostable than spectrally similar dyes
CF®488A	490/516	•	•	•	•	•	DNA-PAINT	• Less non-specific binding than Alexa Fluor® 488
CF®498	498/519	•						• Less non-specific binding than Alexa Fluor® 488 • Use with CF®568/CF®583R and CF®647/Alexa Fluor®647 for multi-color STORM
CF®505	505/519	•						• Identical to ATTO 488
CF®535ST	535/569	•						• Orange dye designed specifically for STORM imaging
CF®555	554/568	• <sup>1</sup>		•				• Brighter and more photostable than Cy®3 • Less non-specific binding than Alexa Fluor® 555
CF®568	562/584	• <sup>1</sup>	•	•		•		• Yields much brighter conjugates vs. Alexa Fluor® 568 • Outperforms Cy®3b in STORM • Pairs well with Biotium's CF®647 and CF®680 in multi-color STORM
CF®583R	585/609	• <sup>1</sup>						• One of two top-performing dyes specifically designed for STORM with green laser (also see CF®597R)
CF®594	593/615		•		•			• Significantly brighter than Alexa Fluor® 594 and Texas Red® • Extremely photostable
CF®597R	597/619	• <sup>1</sup>						• Deep-red fluorescent dye designed for STORM • Top-performing dye specifically designed for STORM with green laser (also see CF®583R)
CF®633	629/650					•	FIONA, gSHRImP, SMT	• Significantly brighter than similar far-red dyes • Far more photostable than Alexa Fluor® 647
CF®640R	642/663		•	•	•	•	FLImP	• Offers improved brightness and photostability over ATTO 647N and spectrally similar dyes
CF®647	652/668	• <sup>1</sup>						• Spectrally similar to Cy®5 and Alexa Fluor® 647 • Pairs well with CF®568 for multi-color STORM • The best far-red dye for demixing-based multi-color (d)STORM imaging when paired with CF®680
CF®660R	662/682					•	SMLM, DNA-PAINT	• Much brighter than Alexa Fluor® 660 • The most photostable 660 nm dye • Validated for use with DNA-PAINT SMLM
CF®660C	667/685	• <sup>1</sup>					MINFLUX	• Much brighter and more photostable than Alexa Fluor® 660 • Ideal for long high-intensity 3D (d)STORM image acquisitions with minimal photobleaching
CF®680	681/698	• <sup>1</sup>					Dual-color 3D SMLM, MINFLUX	• The brightest among spectrally similar 680 nm dyes • Pairs well with CF®568 for multi-color STORM • The best near-IR dye for demixing-based multi-color (d)STORM imaging when paired with CF®647
CF®680R	680/701	• <sup>1</sup>	•		•		Single-molecule spectroscopy, SMT	• The most photostable 680 nm dye • Suitable for labeling nucleic acids and small biomolecules
CF®750	755/779	•						• Exceptionally bright and photostable near-IR dye • Patented pegylated dye for superior performance

<sup>1</sup> Dye was validated in multi-color STORM experiments.

A bullet indicates that the respective dye has been published and/or validated for the application. See our [current list of references](#) validating CF® Dyes for SRM and other applications. For more information regarding SRM validation data, or questions about the compatibility of CF® Dyes with expansion microscopy protocols contact [techsupport@biotium.com](mailto:techsupport@biotium.com).

FLImP: Fluorophore localization imaging with photobleaching; SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastic optical reconstruction microscopy; TIRF: Total internal reflection fluorescence microscopy; FIONA: Fluorescence imaging with one-nanometer accuracy; SMT: Single-molecule tracking; SMLM: Single-molecule localization microscopy.



## Cellular Stains Validated for Super Resolution

Biotium offers a wide selection of novel and classic cellular stains that have been validated for SRM applications. See the table below for a list of cellular stains and validated SRM applications that Biotium offers.

Localization	Catalog Number	Product	Abs/Em (nm)	STORM	STED	SIM	2-Photon*	TIRF
Nucleus	40090	Oxazole Yellow Homodimer (YOYO®-1)	491/509†	•				
	40081	NucSpot® Live 488	503/518			•		
	40082	NucSpot® Live 650	655/681	•	•	•		
Membrane / Cell Surface	30092	MemBrite® Fix 405/430	405/430			•		
	30093	MemBrite® Fix 488/515	488/515		•		•	•
	30095	MemBrite® Fix 568/580	568/580	•		•		•
	30096	MemBrite® Fix 594/615	594/615				•	
	30097	MemBrite® Fix 640/660	640/660			•		•
	30099	MemBrite® Fix 680/700	680/700	•	•		•	
	30101	MemBrite® Fix-ST 650/665	650/665	•				
	30102	MemBrite® Fix-ST 667/685	667/685	•				
	30103	MemBrite® Fix-ST 681/698	681/698	•				
	30104	MemBrite® Fix-ST 755/777	755/777	•				
	30090	CellBrite® Fix 488	480/513			•		
	30088	CellBrite® Fix 555	542/570			•		
	30107	CellBrite® Steady 550	562/579	•				
	30108	CellBrite® Steady 650	656/676	•				
	30109	CellBrite® Steady 685	686/708	•				
	30023	CellBrite® Red	644/665			•	•	•
	70020, 70022	SynaptoGreen™ C4 (FM®1-43)	480/598				•	
Cytoplasm	30050	ViaFluor® CFSE	495/515				•	
Microtubules	70063	ViaFluor® 647	650/675		•	•		
Lysosomes	70067	LysoView™ 488	506/532			•		
	70059	LysoView™ 650	650/675		•	•		
Lipid Droplets	70065	LipidSpot™ 488	427/585			•		
EVs & Exosomes	30115	ExoBrite™ STORM CTB CF®505	505/519	•				
	30116	ExoBrite™ STORM CTB CF®583R	583/609	•				
	30117	ExoBrite™ STORM CTB CF®647	652/668	•				
	30118	ExoBrite™ STORM CTB CF®680	681/698	•				
	<a href="#">Please visit website for more information on catalog numbers and antibody targets.</a>	ExoBrite™ STORM CF®498 Antibodies	498/519	•				
		ExoBrite™ STORM CF®568 Antibodies	562/584	•				
		ExoBrite™ STORM CF®583R Antibodies	585/609	•				
		ExoBrite™ STORM CF®647Plus Antibodies	652/668	•				

\*We also offer a wide selection of ion indicator and cell tracing dyes for use in 2-Photon Microscopy. A bullet indicates that the respective dye has been published and/or validated for the application. For questions regarding the validation of our cellular stains for SRM, please contact [techsupport@biotium.com](mailto:techsupport@biotium.com).

†With DNA

SIM: Structured illumination microscopy; STED: Stimulated emission depletion; STORM: Stochastic optical reconstruction microscopy; TIRF: Total internal reflection fluorescence microscopy.

CY Dye is a registered trademark of Cytiva; Alexa Fluor and Texas Red are registered trademarks, and Prolong and Pacific Blue are trademarks of Thermo Fisher Scientific.



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